Seizure 19 (2010) 237–241

Contents lists available at ScienceDirect

Seizure

journal homepage: www.elsevier.com/locate/yseiz

Association of alpha subunit of $GABA_A$ receptor subtype gene polymorphisms with epilepsy susceptibility and drug resistance in north Indian population

Ritu Kumari [a], Ram Lakhan [a], J. Kalita [b], U.K. Misra [b], Balraj Mittal [a,*]

[a] Department of Genetics, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Raebareli Road, Lucknow 226014, India
[b] Department of Neurology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow 226014, India

ARTICLE INFO

Article history:
Received 5 October 2009
Received in revised form 21 February 2010
Accepted 26 February 2010

Keywords:
$GABA_A$ receptor
Drug resistance
Epilepsy
Antiepileptic drugs
GABRA1 and *GABRG2*

ABSTRACT

GABA (γ-amino butyric acid) receptors have always been an inviting target in the etiology and treatment of epilepsy because of its role as a major inhibitory neurotransmitter in the brain. The aim of our study was to find out the possible role of single nucleotide polymorphisms (SNPs) present in *GABRA1* IVS11 + 15 A > G (rs2279020) and *GABRG2* 588C > T (rs211037) genes in seizure susceptibility and pharmaco-resistance in northern Indian patients with epilepsy. A total of 395 epilepsy patients and 199 control subjects were enrolled for present study. The genotyping was done by PCR-RFLP methods. The *GABRA1* IVS11 + 15 A > G polymorphism conferred high risk for epilepsy susceptibility at genotype 'AG' ($P = 0.004$, OR = 1.77, 95% CI = 1.20–2.63), 'GG' ($P = 0.01$, OR = 1.80, 95% CI = 1.15–2.80) and G allele level ($P = 0.001$, OR = 1.50, 95% CI = 1.16–1.92). Moreover this polymorphism was also associated with multiple drug resistance in patients with epilepsy for homozygous variant 'GG' genotype ($P = 0.031$, OR = 1.84, 95% CI = 1.05–3.23) and G allele ($P = 0.020$, OR = 1.43, 95% CI = 1.05–1.95). However *GABRG2* 588C > T polymorphism was not found to be associated either with epilepsy susceptibility or with drug resistance. Overall results indicate differential role of different subunits of $GABA_A$ receptor subtypes in epilepsy susceptibility and pharmacotherapy.

1. Introduction

Epilepsy is most common paroxysmal and heterogeneous neurological disorder affecting an estimated 42 million people worldwide with distinct symptoms, etiology, prognosis and treatments.[1] Overall prevalence of epilepsy roughly lies in the range of 5–10 per 1000 people, which is usually higher in developing countries.[2,3] Majority of epilepsy phenotypes result from interaction between genes and environmental factors. It is just over a decade since the discovery of the first human epilepsy associated ion channel gene mutation, at least 25 different genes have been described till now, although the strength of the evidences for these genes having a pathogenic role in epilepsy varies. Only 1–2% idiopathic epilepsies seem to be monogenic; whereas most of them are believed to be polygenic.[4] These gene and their variants influence seizures, epileptogenesis and epilepsy at multiple levels.[5] Therefore, genes encoding voltage gated Na^+, K^+, Ca^{++}, Cl^- and HCN,[6,7] and ligand-gated (nicotinic acetylcholine and GABA receptors) ion channels are considered to be major class of genes associated with various epilepsy phenotypes.

In the central nervous system, GABA is the major inhibitory neurotransmitter that controls neuronal excitability and network interactions in the cerebral cortex of the brain. It acts through three receptor classes: the ionotropic $GABA_A$, $GABA_C$ receptors and the metabotropic $GABA_B$ receptors. Among the three receptors, recent findings highlight the significance of $GABA_A$ receptor heterogeneity for the concept of E/I (excitation/inhibition) balance and its relevance for epilepsy.[8] Structurally $GABA_A$ receptors are pentameric chloride ion channels formed from various combinations of proteins encoded by α (α1–α6), β (β1–β3), γ (γ1–γ3); δ, ε, π, θ, and ρ (ρ1–ρ3) subunit gene families. The α1β2γ2 subunit combination of $GABA_A$ receptor is most abundant in almost all regions of the brain.[3] Dysfunction of genes coding these subunits affects ion channel gating, expression, and trafficking of the GABA receptor to the cell surface. These genes are also believed to influence important drug targets necessary for the regulation of neuronal activity in the brain.[9] Antiepileptic drugs (AEDs) such as benzodiazepines, phenobarbital, gabapentin and topiramate are important targets of $GABA_A$ receptor.[10] Recently it has been reported that AED resistant rats differ from drug responsive rats in $GABA_A$ receptor subunit expression in rat model of temporal lobe epilepsy. It also suggests that alterations in $GABA_A$ receptor subunits may be involved in resistance to AEDs.[11]

* Corresponding author. Tel.: +91 522 2668700x2322; fax: +91 522 2668017.
E-mail addresses: balraj@sgpgi.ac.in, bml_pgi@yahoo.com (B. Mittal).

1059-1311/$ – see front matter © 2010 British Epilepsy Association. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.seizure.2010.02.009

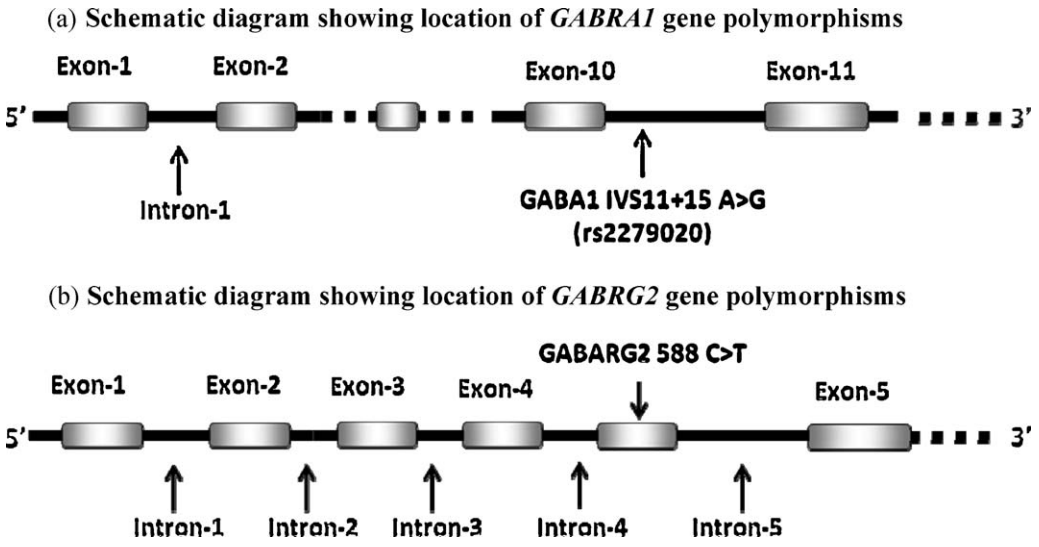

Fig. 1. (a) Schematic diagram showing location of *GABRA1* gene polymorphisms. (b) Schematic diagram showing location of *GABRG2* gene polymorphisms.

Several SNPs in the $GABA_A$ receptor subtypes have been described so far but only few including intronic *GABRA1* IVS11 + 15 A > G and an exonic *GABRG2* 588C > T gene polymorphisms are found to have functional significance in different neurological disorders. These gene variants have been attributed as one of the several susceptibility factors for febrile seizures[9,12]; with the development of alcoholism and substance abuse disorders affecting neuronal channels.[13,14]

Thus, genes encoding $GABA_A$ receptor subunits represent high ranking candidates for epilepsy susceptibility and targets for pharmacotherapeutic agents in epilepsy treatment. Therefore, on the basis of functional significance, previous observations and current knowledge we investigated the possible role of these genetic polymorphisms *GABRA1* IVS11 + 15 A > G (rs2279020) and *GABRG2* 588C > T (rs211037) [Fig. 1(a) and (b)] in epilepsy susceptibility and antiepileptic drug (AED) response in northern Indian patients with epilepsy.

2. Materials and methods

2.1. Patients and controls

Epilepsy patients were enrolled from the outpatients department (OPD) of neurology attending the clinics of Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGIMS), Lucknow, India. The patients were diagnosed and classified by an experienced neurologist. The clinical profile of drug responsive and drug resistant epilepsy patients were based on hospital investigations. Exclusion criteria included severe adverse drug reactions; poor compliance with AEDs, unreliable record of seizure frequency, history of pseudo seizures, alcohol or drug abuse, or any other malignant diseases such as brain tumor, secondary metastasis, hepatic failure or renal failure. An informed consent was signed by each participant or responsible adult and they were personally interviewed for information on ethnicity, seizure frequency, and duration of seizure, compliance and other habits. After screening of more than 500 patients a total of 395 patients were included rest were excluded. 259 patients were diagnosed as drug responsive and 122 are nonresponsive. We found that in responders group, 123 (45.1%) patients were on monotherapy and 150 (54.9%) were on polytherapy, i.e. on more than two drugs. In case of nonresponsive epilepsy patients, all were undergoing polytherapy. It was observed that patients who respond early to treatment are less likely to become drug resistant. There appears to be no significant differences in response when compared on the basis of monotherapy and polytherapy. Fourteen patients showed only partial response and therefore excluded from the study analysis involving drug response.

A total of 199 healthy controls were recruited from staff of SGPGIMS and unrelated persons from north India visiting the hospital for minor medical or surgical problems, reported no history of epileptic seizures, and other brain abnormalities. All controls, drug resistance and drug responsive patients were of same ethnic origin. The study was approved by local ethics committee of the institute at SGPGIMS, Lucknow, India.

2.2. Definition of drug resistance and responsiveness

The main criterion for drug resistance was the occurrence of at least four seizures over a period of one year with three appropriate antiepileptic drugs (AEDs) at maximum tolerated doses.[15,16] Patients who had under-gone surgeries for seizure control were considered refractory irrespective of their outcome after surgery. The epilepsy patients who had complete freedom from seizures for at least one year from last follow up visit were considered drug responsive.

In order to ascertain drug compliance, antiepileptic drug levels in plasma were measured using HPLC (Perkin Elmer) in 20% of patients to confirm compliance and all patients enrolled in the study showed drug compliance. Mean carbamazepine, phenytoin and valproate levels were 8.26 ± 5.25 μg/ml, 11.27 ± 8.12 μg/ml and 68.0 ± 36.22 μg/ml respectively in epileptic patients; and were in therapeutic range. The maximum tolerated doses were different for different individuals in our epilepsy patients. These were 20 mg/kg/day for carbamazepine, 20 mg/kg for phenytoin and 10 mg/kg for valproate.

2.3. Laboratory protocols

2.3.1. Genotyping of GABRA1 (rs2279020) and GABRG2 (rs211037)

The genomic DNA was extracted from peripheral blood leucocytes pellet using the standard salting out method with slight modifications.[17] The plasma was separated and stored at −20 °C for drug level assay. We genotyped total 395 epilepsy patients and 199 healthy controls. Genotyping was performed using PCR-RFLP method as reported previously (Table 1). Twenty percent of samples from patients including samples of each genotype were re-genotyped by different laboratory personnel and

Table 1
List of primers used.

SNP	Primer sequence	Restriction enzyme	Reference
GABRA1 IVS11 + 15 A > G (rs2279020)	F 5′-GCT ATG GAT TGG TTT ATT GCC GTG TG-3′ R 5′-ATA ATA TTG ATG TAC TAC AGG GAC-3′	AvaII	14
GABRG2 588C > T (rs211037)	F 5′-AATCACCTTTTATTCTAATGGTC-3′ R 5′-CAGTGAAGGCAACTTACTAAGA-3′	ApoI	9

results were concordant with no discrepancy noticed in genotyping. PCR reaction was carried out in final volume of 20 μl containing 50–100 ng genomic DNA. PCR conditions were as follows: a denaturing step at 95 °C for 5 min, then 30 cycles at 94 °C for 30 s, annealing temperature 60 °C for *GABRA1* IVS11 + 15 and 57 °C for *GABRG2* 588C > T for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 7 min. After amplification, PCR products of 165 bp and 122 bp observed respectively; which were digested using specific restriction endonuclease. The AvaII (Fermentas Inc., USA) RFLP assay in *GABRA1* IVS11 + 15 A > G polymorphism was used to distinguish the A/G substitution at nucleotide 15 of the last intron and the products were separated by using 15% poly-acryl amide gel electrophoresis and genotyping patterns were recorded. For *GABRA1* IVS11 + 15 polymorphism genotypes were classified as 'AA' homozygote 165 bp, 'AG' heterozygote 165 bp and 141 bp, and 'GG' homozygote 141 bp (Fig. 2). The *GABRG2* 588C > T polymorphism containing PCR product of 122 bp was digested with ApoI restriction enzyme (NEB Inc., USA) at 57 °C in total volume of 10 μl. After restriction digestion with ApoI; the T allele produced two fragments of 102 bp and 20 bp and stained with ethidium bromide (Fig. 3). Gel documentation was done using Alpha imager™ 1220, Alpha Innotech Corporation, and San Leandro, CA.

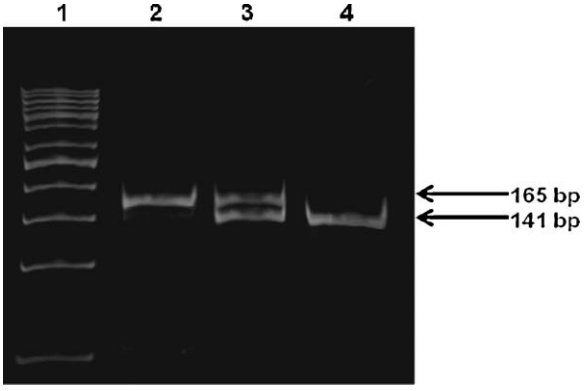

Fig. 2. Representative gel picture for *GABRA1* IVS11 + 15 A > G (rs2279020) genotyping; lane 1, 50 bp DNA ladder; lane 2, homozygous AA genotype; lane 3, heterozygous AG genotype; lane 4, homozygous GG genotype.

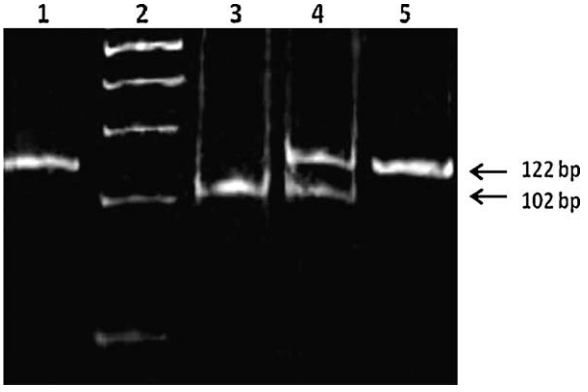

Fig. 3. Representative gel picture for *GABRG2* 588C > T (rs211037) genotyping. Lane 1, undigested; lane 2, DNA ladder; lane 3, homozygous CC genotype; lane 4, heterozygous CT genotype; lane 5, homozygous TT genotype.

2.4. Statistical analysis

The relationship between various genotypes and responsiveness was examined using binary logistic regression. Association was expressed as odds ratios (OR) or risk estimates with 95% confidence intervals (CI). The significant association was considered when *P*-value was <0.05. All analyses were performed using the SPSS statistical analysis software, version 15.0 (SPSS, Chicago, IL, USA). The sample size was calculated using the QUANTO 1.1 program (http://hydra.usc.edu/gxe). Desired power of study was set 80%. Relative risks for power calculation were set at 2.

3. Results

The mean age ± SD of patients with epilepsy was 24.25 ± 11.65. Among all the patients with epilepsy, 72.2% (285/395) were male and 27.8% (110/395) were female. Patients were also categorized on the basis of their drug response status. The mean age of the drug resistant patients was 23.84 ± 11.94 years versus drug responsive patients was 24.72 ± 11.24 years. Epilepsy was classified as symptomatic, idiopathic, or cryptogenic. Seizures were classified as generalized or partial; 57.5% (227/395) had generalized seizures, whereas 42.5% (168/395) had partial seizures. Mean age of onset for seizures in epilepsy patients was 16.39 ± 10.71 years. We were not able to correlate drug levels in patients with genotypes because sample size was very small for the given drugs and particular genotypes.

Among responders 116 (44.8%) were found to have symptomatic and 143 (55.2%) idiopathic epilepsy. Similarly among drug resistant group 58 (47.5%) were having symptomatic epilepsy and 64 (52.5%) exhibited idiopathic epilepsy.

3.1. Association of GABRA1 A > G and GABRG2 588C > T gene polymorphisms and susceptibility to epilepsy

We analyzed the genotype and allelic frequencies in 395 sporadic epilepsy patients and 199 control subjects by PCR-RFLP assay (Tables 2 and 3). Genotype and allelic frequencies of *GABRA1* IVS11 + 15 A > G and *GABRG2* 588C > T polymorphisms studied were consistent with Hardy–Weinberg equilibrium ($P = 0.730$) in our control population. Frequencies of AG and GG genotypes of *GABRA1* IVS11 + 15 A > G were found to be significantly higher in epilepsy patients versus control subjects for AG ($P = 0.004$, OR = 1.77, 95% CI = 1.20–2.63), GG ($P = 0.010$, OR = 1.80, 95% CI = 1.15–2.80) genotype as well as for variant G allele ($P = 0.001$, OR = 1.50, 95%, CI = 1.16–1.92) (Table 2). However, for *GABRG2* 588C > T gene polymorphism we did not observe any significant differences in genotype or allelic frequencies between the epilepsy and control subjects (Table 3).

After Bonferroni correction for multiple testing, reference *P*-value became 0.01 where it was 0.001 (Table 2 for 'G' allele); and for AG genotype for which *P*-value became 0.04, in both cases it is

Table 2
Distribution of *GABRA1* (rs2279020) polymorphism in epilepsy patients versus healthy controls and drug responsive versus drug resistant epilepsy.

Genotypes/ allele	Control subjects (*N* = 199)	Epilepsy patients (*N* = 395)	*P*-Value	OR (95% CI)	Drug responsive (*N* = 259)	Drug resistant (*N* = 122)	*P*-Value	Odds ratio (95% CI)
AA	94 (47.23%)	132(33.41%)	Reference	Reference	92(35.52%)	34 (27.86%)	Reference	Reference
AG	63(31.65%)	157(39.74%)	0.004	1.77(1.20–2.63)	107(41.31%)	47 (38.52%)	0.517	1.18(0.70–2.00)
GG	42(21.10%)	106(26.83%)	0.010	1.80(1.15–2.80)	60(23.16%)	41(33.60%)	0.031	1.84(1.05–3.23)
A*	251 (63.06%)	421 (53.29%)	Reference	Reference	291(56.18%)	115(47.13%)	Reference	Reference
G*	147 (36.93%)	369 (46.71%)	0.001	1.50(1.16–1.92)	227(43.82%)	129(52.87%)	0.020	1.43(1.05–1.95)

Table 3
Distribution of *GABRG2* (rs211037) polymorphism in epilepsy patients versus healthy controls and responsive versus refractory epilepsy.

Genotypes/ allele	Healthy controls (*N* = 199)	Epilepsy patients (*N* = 395)	*P*-Value	Odds ratio (95% CI)	Drug responsive (*N* = 259)	Drug resistant (*N* = 122)	*P*-Value	Odds ratio (95% CI)
CC	117(58.79%)	211(53.41%)	Reference	Reference	137(52.89%)	66 (54.09%)	Reference	Reference
CT	73(36.68%)	168(42.53%)	0.179	1.28 (0.89–1.82)	109(42.08%)	53 (43.44%)	0.967	1.01(0.65–1.57)
TT	9(4.52%)	16(4.05%)	0.974	0.99 (0.42–2.30)	13(5.01%)	3(2.45%)	0.263	0.48(0.13–1.74)
C*	307 (77.13%)	591(74.68%)	Reference	Reference	383(73.94%)	185(75.81%)	Reference	Reference
T*	91 (22.86%)	199(25.31%)	0.379	1.14 (0.85–1.51)	135(26.06%)	59(24.18%)	0.578	0.91(0.64–1.29)

less than 0.05 (reference *P*-value) and statistically significant while in case of GG genotype significance disappeared after Bonferroni correction.

3.2. GABRA1 (rs2279020) and GABRG2 (rs211037) polymorphism in drug resistance epilepsy

We also observed significant difference at genotype as well as allele frequencies of *GABRA1* A > G polymorphism in drug resistant versus drug responsive epilepsy patients for homozygous variant GG genotype ($P = 0.031$, OR = 1.84, 95% CI = 1.05–3.23) and G allele ($P = 0.020$, OR = 1.43 95% CI = 1.05–1.95, Table 2). However, in *GABRG2* 588C > T we did not observe any significant differences in drug resistant versus drug responsive epilepsy patients either at genotype or allele levels (Table 3). It suggests *GABRA1* IVS11 + 15 A > G polymorphism modulates drug response as well as susceptibility for epilepsy in north Indian epilepsy patients.

4. Discussion

In the present study, we found the involvement of *GABRA1* IVS11 + 15 A > G polymorphism in increasing risk for developing epilepsy as well as in modulating drug response in pharmacotherapy, while *GABRG2* 588C > T was not found to be associated either with epilepsy susceptibility or with drug resistance in north Indian epilepsy subjects.

Upon comparing allele and genotype frequencies of variant of *GABRA1* IVS11 + 15 A > G in our control subjects with that reported for Chinese and Japanese population in Hapmap project data; similar patterns were observed. Moreover, allele frequency of this polymorphism was also similar to that reported in an Indian population of Gujarati individuals (a state in western India) living in Houston, TX (http://www.hapmap.org/cgi-perl/gbrowse/hapmap3B36/). However, allele and genotype frequencies of *GABRG2* 588C > T polymorphism show wide variation across different world populations[9,18] suggesting role of ethnic differences in distribution of genetic variants. We minimized the influence of genetic heterogeneity by inclusion of subjects from north India and distribution of genotypes in our control population for both the polymorphisms were consistent with Hardy–Weinberg equilibrium (HWE).

To the best of our knowledge, this is the first report in which an association of *GABRA1* IVS11 + 15 A > G polymorphism was observed with epilepsy susceptibility and drug resistance. This is an intronic polymorphism which does not lead to any amino acid change but it is possible that it alters the conformation of mature protein by influencing alternative splicing. Several mutations in this gene have also been reported to be involved in epilepsy causation that result in loss of function of $GABA_A$ receptors via a reduction in GABA expression and, accelerated deactivation.[19]

Another most widely studied single nucleotide polymorphisms (SNPs) in the human $GABA_A$ receptor is *GABRG2* 588C > T gene polymorphism present at position 588 of exon 5. It results in synonymous or silent change asn196asn that does not affect the sequence of the encoded protein, suggesting that this SNP exists in linkage disequilibrium with other disease causing variants. An earlier study has reported association of this synonymous variant with febrile seizures in a cohort of 104 Taiwanese children [$P = 0.017$, OR = 2.56, 95% CI = 1.01–6.50].[9] However, we did not find association of this polymorphism either with epilepsy

Table 4
GABRG2 588 C > T (rs211037); association studies in seizure disorder.

Phenotypes	No. cases	No. controls	Country of origin	*P*-Value	Reference
Febrile convulsions	74	118	USA	0.8	23
Generalized	77	83	Taiwan	0.002	9
All epilepsy	1361	656	UK and Ireland	>0.11	24
Generalized	99	364	UK	0.61	
Generalized	121	284	Ireland	0.95	
Febrile convulsion	104	83	Taiwan	0.009	20
Febrile Convulsions	94	106	Japan	0.5	25
Generalized	135	154	Germany	0.5	18
Generalized	58	58	China	0.07	26
Febrile Convulsion	107	384	UK	0.2	27
Generalized	96	384	UK	0.6	
All epilepsy	395	199	India	0.37	Present study
Generalized	227	199	India	0.519	

susceptibility or drug resistance. In our study, the number of patient with febrile seizures was limited in our study subjects and etiology of other sporadic epilepsies is different from febrile seizure. Similarly studies in Caucasian and other populations also failed to replicate its association in epilepsy patients (Table 4) except in Taiwanese population.[9,20] It suggests that association of this polymorphism is population specific.

It is now well established that various AEDs mediate their action through GABA binding.[21] It is also hypothesized that target receptor sites are somehow altered in the epileptic brain so that they are much less sensitive to administered AEDs. Therefore, it is possible that the association of *GABRA1* IVS11 + 15 A > G polymorphism with refractory phenotype in our study may occur due to changes in the structure and function of inhibitory $GABA_A$ receptors. Excessive glutamate excitation and activation of drug resistance genes may also contribute to changes in GABA receptor conformation and loss of drug efficacy.

Overall, results from our study suggest differential behavior of subunits of $GABA_A$ receptor gene polymorphisms in epilepsy susceptibility and its therapy. However, in addition to genetic factors, there are multiple causes of drug resistance in epilepsy that include past treatment history[22] and other clinical factors such as type of epilepsy, duration of seizure, and number of seizures prior to initiation of drug therapy.

Thus, our findings are supportive of the fact that different subunits of $GABA_A$ receptor subtypes play differential roles in epilepsy at multiple levels and may affect inter-individual variation in drug response for AEDs used in the treatment of epilepsy. Till now very few studies have explored role of these genetic variants in epilepsy and multiple drug resistance; it would be desirable to study them at functional level and also to replicate them in larger cohorts.

Acknowledgments

The study was supported by a grant received from the Department of Biotechnology, Government of India, and fellowships provided by CSIR and DST (New Delhi), India. We thankfully acknowledge support from Dr. NJ Gogtay, KEM Hospital Mumbai, for drug level assay.

References

1. Depondt C. The potential of pharmacogenetics in the treatment of epilepsy. *Eur J Paediatr Neurol* 2006;**10**:57–65.
2. Helbig I, Scheffer IE, Mulley JC, Berkovic SF. Navigating the channels and beyond: unravelling the genetics of the epilepsies. *Lancet Neurol* 2008;**7**:231–45.
3. Reid CA, Berkovic SF, Petrou S. Mechanisms of human inherited epilepsies. *Prog Neurobiol* 2009;**87**:41–57.
4. Weber YG, Lerche H. Genetic mechanisms in idiopathic epilepsies. *Dev Med Child Neurol* 2008;**50**:648–54.
5. Sisodiya SM. Genetics of drug resistance in epilepsy. *Curr Neurol Neurosci Rep* 2005;**5**:307–11.
6. Lakhan R, Kumari R, Misra UK, Kalita J, Pradhan S, Mittal B. Differential role of sodium channels *SCN1A* and *SCN2A* gene polymorphisms with epilepsy and multiple drug resistance in north Indian population. *Br J Clin Pharmacol* 2009;**68**:214–20.
7. Lu Y, Wang X. Genes associated with idiopathic epilepsies: a current overview. *Neurol Res* 2009;**31**:135–43.
8. Fritschy JM. Epilepsy, E/I balance and GABA(A) receptor plasticity. *Front Mol Neurosci* 2008;**1**:5.
9. Chou IC, Lee CC, Tsai CH, Tsai Y, Wan L, Hsu YA, et al. Association of GABRG2 polymorphisms with idiopathic generalized epilepsy. *Pediatr Neurol* 2007;**36**:40–4.
10. Moshe SL. Mechanisms of action of anticonvulsant agents. *Neurology* 2000;**55**:S32–40. discussion S54–38.
11. Bethmann K, Fritschy JM, Brandt C, Loscher W. Antiepileptic drug resistant rats differ from drug responsive rats in GABA A receptor subunit expression in a model of temporal lobe epilepsy. *Neurobiol Dis* 2008;**31**:169–87.
12. Kang JQ, Macdonald RL. Making sense of nonsense GABA(A) receptor mutations associated with genetic epilepsies. *Trends Mol Med* 2009;**15**:430–8.
13. Czuczwar SJ. GABA-ergic system and antiepileptic drugs. *Neurol Neurochir Pol* 2000;**34**(Suppl. 1):13–20.
14. Park CS, Park SY, Lee CS, Sohn JW, Hahn GH, Kim BJ. Association between alcoholism and the genetic polymorphisms of the GABAA receptor genes on chromosome 5q33–34 in Korean population. *J Korean Med Sci* 2006;**21**:533–8.
15. Siddiqui A, Kerb R, Weale ME, Brinkmann U, Smith A, Goldstein DB, et al. Association of multidrug resistance in epilepsy with a polymorphism in the drug-transporter gene ABCB1. *N Engl J Med* 2003;**348**:1442–8.
16. Lakhan R, Misra UK, Kalita J, Pradhan S, Gogtay NJ, Singh MK, et al. No association of ABCB1 polymorphisms with drug-refractory epilepsy in a north Indian population. *Epilepsy Behav* 2009;**14**:78–82.
17. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;**16**:1215.
18. Kananura C, Haug K, Sander T, Runge U, Gu W, Hallmann K, et al. A splice-site mutation in GABRG2 associated with childhood absence epilepsy and febrile convulsions. *Arch Neurol* 2002;**59**:1137–41.
19. Krampfl K, Maljevic S, Cossette P, Ziegler E, Rouleau GA, Lerche H, et al. Molecular analysis of the A322D mutation in the GABA receptor alpha-subunit causing juvenile myoclonic epilepsy. *Eur J Neurosci* 2005;**22**:10–20.
20. Chou IC, Peng CT, Huang CC, Tsai JJ, Tsai FJ, Tsai CH. Association analysis of gamma 2 subunit of gamma-aminobutyric acid type A receptor polymorphisms with febrile seizures. *Pediatr Res* 2003;**54**:26–9.
21. Benarroch EE. GABAA receptor heterogeneity, function, and implications for epilepsy. *Neurology* 2007;**68**:612–4.
22. Schiller Y, Najjar Y. Quantifying the response to antiepileptic drugs: effect of past treatment history. *Neurology* 2008;**70**:54–65.
23. Ma S, Abou-Khalil B, Blair MA, Sutcliffe JS, Haines JL, Hedera P. Mutations in GABRA1, GABRA5, GABRG2 and GABRD receptor genes are not a major factor in the pathogenesis of familial focal epilepsy preceded by febrile seizures. *Neurosci Lett* 2006;**394**:74–8.
24. Kinirons P, Cavalleri GL, Shahwan A, Wood NW, Goldstein DB, Sisodiya SM, et al. Examining the role of common genetic variation in the [gamma]2 subunit of the GABAA receptor in epilepsy using tagging SNPs. *Epilepsy Res* 2006;**70**:229–38.
25. Nakayama J, Hamano K, Noguchi E, Horiuchi Y, Iwasaki N, Ohta M, et al. Failure to find causal mutations in the GABA(A)-receptor gamma2 subunit (GABRG2) gene in Japanese febrile seizure patients. *Neurosci Lett* 2003;**343**:117–20.
26. Lu J, Chen Y, Zhang Y, Pan H, Wu H, Xu K, et al. Mutation screen of the GABA(A) receptor gamma 2 subunit gene in Chinese patients with childhood absence epilepsy. *Neurosci Lett* 2002;**332**:75–8.
27. Cavalleri GL, Lynch JM, Depondt C, Burley MW, Wood NW, Sisodiya SM, et al. Failure to replicate previously reported genetic associations with sporadic temporal lobe epilepsy: where to from here? *Brain* 2005;**128**:1832–40.